



AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Currently amended) A method of enumerating the number of CD4+ (CD4-positive) lymphocytes in a cell sample, the method comprising the steps of:

a) counting the number of white blood cells in a cell sample on a haematology analyser or flow cytometrically by counting total CD45+ expressing cells~~identifying the total white blood cell population as a reference population from which the CD 4+ lymphocytes are subsequently measured;~~

b) determining flow cytometrically the proportion or the percentage of white blood cells counted in step (a) that are CD4+ lymphocytes as a function of the total white blood cell reference population in a sample identified in step (a), by:

(i) establishing a primary gate to identify all CD45 expressing white blood cells;

(ii) within this primary gate, identifying a secondary gate in which the CD4+ lymphocytes are identified by their bright CD4++/low side scatter expression; and

(iii) expressing these CD4 lymphocytes as a percentage or proportion of the total CD45 expressing white blood cells identified in the primary gate;

~~c) determining the number of white blood cells per volume of blood in the sample; and~~

~~d)c) calculating the absolute number of CD4+ lymphocytes in the cell sample by multiplying the proportion or percentage of white blood cells that are CD4+ lymphocytes, obtained in step (b), by the number of white blood cells counted obtained in step (ca).~~

2. (Currently amended) A method according to claim 1, wherein the number of CD4+ lymphocytes is enumerated in a single platform approach whereby the number of white blood cells counted in step (a) is obtained on a flow cytometer

using a bead-based or volumetric-based counting method and the proportion or percentage of CD4+ lymphocytes determined in step (b) is also performed on a flow cytometer, or whereby the number of white blood cells counted in step (a) is obtained on a haematology analyser and the proportion or percentage of CD4+ lymphocytes determined in step (b) is also performed on a haematology analyser
~~white blood cells per volume of blood is determined in step (c) by a single platform determination of total CD45+ expressing cells.~~

3. (Withdrawn) A method according to claim 1, wherein a white blood cell differential is further identified from the total CD45 expressing population identified in step (a) of claim 1, and further comprising the steps of:

- e) (i) determining the percentage of CD4 monocytes as a function of the total CD45 expressing population identified in step (a); and
- (ii) calculating the number of CD4 monocytes in the sample by multiplying the percentage of CD4 monocytes determined in step (e(i)) by the number of CD45 cells obtained in step (c);
- f) (i) determining the percentage of CD4 eosinophils as a function of the total CD45 expressing population identified in step (a); and
- (ii) calculating the number of CD4 eosinophils in the sample by multiplying the percentage of CD4 eosinophils determined in step (f(i)) by the number of CD45 cells obtained in step (c);
- g) (i) determining the percentage of CD4 negative granulocytes as a function of the total CD45 expressing population identified in step (a); and
- (ii) calculating the number of CD4 negative granulocytes in the sample by multiplying the percentage of CD4 negative granulocytes determined in step (g(i)) by the number of CD45 cells obtained in step (c);

- h) (i) determining the percentage of CD4 negative lymphocytes as a function of the total CD45 expressing population identified in step (a); and
- (ii) calculating the number of CD4 negative lymphocytes in the sample by multiplying the percentage of CD4 negative lymphocytes determined in step (h(i)) by the number of CD45 cells obtained in step (c);
- i) (i) adding the percentage of CD4 lymphocytes identified in step (b) and the percentage of CD4 negative lymphocytes obtained in step (h(i)) to obtain the percentage of total lymphocytes;
- (ii) determining the percentage of total lymphocytes as a function of the total CD45 expressing population identified in step (a); and
- (iii) calculating the number of total lymphocytes in the sample by multiplying the percentage of total lymphocytes obtained in step (i(ii)) by the number of CD45 cells obtained in step (a).

4. (Withdrawn) A method according to claim 1, further comprising the steps of:

- e) determining the percentage of basophils as a function of the total CD45 expressing population identified in step (a); and
- f) calculating the number of basophils in the sample by multiplying the percentage of basophils determined in step (j) by the number of CD45 cells obtained in step (c).

5. (Previously presented) A method according to claim 1, wherein the sample is whole unlysed blood, unfractionated, fractionated or lysed whole blood.

6. (Withdrawn) A kit including CD4 and CD45 antibodies for use in enumerating the number of CD4 cells in a sample.

7. (Withdrawn) A kit according to claim 6, which further includes instructions for performing the method of enumerating the number of CD 4 cells in a cell sample.

8. (Withdrawn) A kit according to claim 6, which further includes one or more reagents selected from the group consisting of a red cell lysating agent, a stabilizer, a fixative, control cells, media and bead reagents.

9. (Withdrawn) A machine readable medium comprising instructions, which when executed by a machine, cause the machine to perform the method steps of claim 1.

10. (Withdrawn) A machine readable medium according to claim 9, which is configured for use in conjunction with a flow cytometer and/or haematology analyser.

11. (Withdrawn) A machine readable medium according to claim 9, which includes instructions for performing analysis methods selected from the group consisting of impedance, light scatter, fluorescence and precision volume counting.

12. (Withdrawn) The method of claim 1, wherein the method includes the step of enumerating the number of CD4+ lymphocytes or CD4+ T-cells in a cell sample from the patient with HIV or other immune deficiency condition or disease; and wherein the method is used to monitor the immune status of the patient.

13. (Withdrawn) A method according to claim 12, wherein the patient's immune status is determined or monitored to determine the patient's response to antiretroviral treatment.

14. (Previously presented) A method according to claim 2, wherein the single platform determination is performed by adding known numbers of beads to the

sample and counting the beads and cells simultaneously to obtain the absolute cell count.

15. (Currently amended) A method according to claim 1, wherein the number of white blood cells per volume of blood is determined in step (c) by CD4+ lymphocytes is enumerated in a dual platform determination using a haematology analyzer derived total white blood cell count approach whereby the number of white blood cells counted in step (a) is obtained from a haematology analyser and the proportion or percentage of CD4+ lymphocytes determined in step (b) is performed on a flow cytometer.

16. (Previously Presented) A method according to claim 1, wherein the number of white blood cells per volume of blood is determined in step (c) by determination of total CD45+ expressing cells.